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Accepted Article

New advances in the molecular classification of pediatric mesenchymal tumors

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ABSTRACT

Pediatric soft tissue tumors are relatively rare and show significant overlap in morphology and immunoprofile, often posing diagnostic and management challenges. Thus their classification remains often subjective or lumped under ‘unclassified categories’, as a number of lesions lack objective and reproducible criteria in diagnosis. Although in a subset of cases immunohistochemistry has been proved useful to identify a specific line of differentiation, most tumors lack a readily defined histogenesis, being characterized by a rather non-specific immunoprofile. Furthermore, tumors with an ambiguous diagnosis are difficult to grade and their risk of malignancy or clinical management remains uncertain. Advances in molecular genetics, including the more wide application of next generation sequencing in routine clinical practice, have improved diagnosis and refined classification based on objective molecular markers. Importantly, some soft tissue tumors in children are characterized by recurrent gene fusions involving either growth factors (e.g. PDGFB) or protein kinases (e.g. ALK, ROS, NTRK, BRAF), which have paved the way for new targeted treatments that block the respective upregulated downstream pathways. However, the majority of gene fusions or mutations detected in soft tissue tumors result in an abnormal function of transcription factors or chromatin remodeling.¹ The present review focuses on the latest genetic discoveries in the spectrum of both benign and malignant pediatric soft tissue neoplasia. These genetic abnormalities promise to provide relevant insight for their proper classification, prognosis, and treatment. The entities discussed herein are grouped either based on their shared genetic mechanism or based on their presumed line of differentiation.

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I. PEDIATRIC MESENCHYMAL TUMORS DRIVEN BY RECURRENT KINASE FUSIONS

1. INFANTILE FIBROSARCOMA AND RELATED ENTITIES.

A. Infantile Fibrosarcoma (IFS) with canonical ETV6-NTRK3 fusions. IFS is a childhood sarcoma that typically presents at birth or in the first year of life.² IFS has a variable anatomic distribution, with presentation in the distal upper or lower extremities, but also in the trunk and head and neck area. Compared to other high-grade childhood sarcomas, IFS has a favorable outcome, with conservative surgical resection being the preferred therapy, in particular when aiming for limb salvage. Although cytotoxic chemotherapy has shown activity in IFS, its use is limited to selective cases, unamenable to surgery. Only recently, targeted therapy with Larotrectinib, a selective NTRK inhibitor, has been administered in advanced stage IFS with promising results.³ Histologically, IFS is a monomorphic spindle cell sarcoma composed of mitotically active, immature fibroblastic spindle cells arranged in cellular sheets and fascicles, sometimes with prominent lymphocytic infiltrate and hemangiopericytoma-like vascular pattern. The immunoprofile of IFS is rather nonspecific, with only a small subset showing focal expression of SMA or CD34. IHC using a Pan-TRK rabbit monoclonal antibody has proven to be a sensitive but not completely specific marker for IFS.⁴

B. IFS-like lesions with related kinases fusions. A subset of childhood sarcomas that strongly resemble IFS by morphologic criteria (IFS-like sarcomas) harbor recurrent chromosomal abnormalities other than *ETV6-NTRK3*, including *EML4-NTRK3* variant fusions and rearrangements of the kinase genes *ALK*, *BRAF* and *NTRK1* (Fig 1), summarized in **Table 1**.⁵⁻⁷ Most IFS-like sarcomas occur in children under age 2, but some present at older age with a predilection for intra-abdominal sites. Clinical outcome is less predictable with some cases showing aggressive clinical behavior, including distant metastases.

More recently a few cases of IFS have been reported to demonstrate compound intragenic *BRAF* deletions associated with tandem duplication of exon 2.⁸ Their mechanism of activation is most likely similar to the *BRAF*-related fusions, resulting in loss of the N-terminal of BRAF protein containing the negative regulatory Ras-binding domain (RBD), with subsequent constitutive activation of BRAF. Interestingly, in that study, 2 of 3 IFS with *BRAF* intragenic deletions show coexisting *ETV6-NTRK3* fusions. Further investigations are needed to clarify if these genetic abnormalities are subclonal or work synergistically in tumorigenesis, and if they might be related to resistance to targeted therapy.

An additional case with morphologic features of IFS but unusual S100 expression was recently reported by our group in a 4-month-old girl with a large, locally aggressive pelvic mass.⁹ By whole transcriptome sequencing the tumor showed a novel *TFG-MET* fusion, retaining the MET kinase domain. The tumor was composed of cellular fascicles with monomorphic hyperchromatic spindle cells that displayed a high mitotic rate, a histomorphology resembling IFS. Moreover, the

tumor showed strong and diffuse S100 expression, whereas SOX10 was negative and H3K27me3 retained, an immunoprofile inconsistent with a diagnosis of malignant peripheral nerve sheath tumor (MPNST).

C. Cellular mesoblastic nephroma – shared genetics with IFS.

Congenital cellular mesoblastic nephroma (CMN), a renal tumor presenting in children during the first months of life, often harbours the same recurrent *ETV6-NTRK3* fusion found in the majority of IFS.¹⁰ Compared to translocation-negative variants of congenital mesoblastic nephroma with classic or mixed histology, the cellular variant with *ETV6-NTRK3* fusion carries a far better prognosis in terms of relapse free and overall survival.¹¹ Some of the genetic alterations found in pediatric IFS-like sarcomas have also been described in subsets of cellular mesoblastic nephroma, including *EML4-NTRK3*⁵, *LMNA-NTRK1*⁵, and *BRAF* internal duplications⁸, providing further evidence that IFS and CMN are histogenetically related entities.

2. LIPOFIBROMATOSIS-LIKE NEURAL TUMORS AND RELATED ENTITIES

Lipofibromatosis-like neural tumor (LPF-NT) is a recently defined entity based on its recurrent *NTRK1* gene fusions. The descriptive designation, LPF-NT, was used to emphasize its close resemblance to lipofibromatosis and its incomplete neural immunophenotype with S100 positivity but negative SOX10.^{12,13} The tumors present in children and young adults as superficial, infiltrative lesions in the extremities. A recent case presenting in an infant as a pigmented back lesion was recently reported.¹⁴ Microscopically, LPF-NT are characterized by monomorphic spindle cells, haphazardly arranged in a reticular pattern and diffusely infiltrating into the subcutaneous fat (Fig. 2). Tumor cells may show a mild degree of nuclear atypia and rare mitoses. By IHC, LPF-NT show expression of S100 and variable positivity for CD34. This immunoprofile might suggest an alternative peripheral nerve sheath tumor diagnosis, however, tumors consistently lack SOX10 expression. Molecularly, LPF-NT harbor activating *NTRK1* fusions, common gene partners including *LMNA*, *TPR*, and *TPM3*, resulting mostly from intra-chromosomal interstitial deletions or inversions.¹² As a result, tumors express NTRK1 (Fig. 2), as shown by IHC with either NTRK1 or pan-TRK antibodies.

The differential diagnosis of LPF-NT also includes lipofibromatosis (LPF) and dermatofibrosarcoma protuberans (DFSP). LPF is a childhood tumor, occasionally present at birth, which has a predilection for hands and feet. Microscopically, it is composed of intersecting long and thin fascicles of fibroblastic spindle cells that infiltrate subcutaneous fat or skeletal muscle, a pattern reminiscent of infantile-type fibromatosis. LPF lacks the nodular fibromyxoid areas, typically seen in the triphasic morphology of fibrous hamartoma of infancy.¹⁵ LPF shows variable expression of CD34 and focal S100 staining, but lacks NTRK1 and beta-catenin reactivity. Genetic studies to date have not revealed recurrent abnormalities, including no *NTRK1* fusions.¹²

Pediatric DFSP is rare and typically occurs under a variant morphologic phenotype, described as giant cell fibroblastoma. These lesions may mimic both LPF and LPF-NT, due to its honeycomb infiltrative growth within subcutis and CD34 reactivity. However, these lesions are consistently negative for S100 and harbor in nearly all cases a recurrent *COL1A1-PDFGB* fusion, resulting in autocrine activation of PDGFRB, which can be targeted with imatinib therapy in advanced/inoperable cases.¹⁶

3. INFLAMMATORY MYOFIBROBLASTIC TUMORS HARBORING FUSIONS INVOLVING ALK AND OTHER KINASES

About half of inflammatory myofibroblastic tumors (IMTs) regardless of anatomic location harbor *ALK* gene rearrangements and overexpress ALK protein. IMTs have a predilection for lung, viscera, and soft tissue sites and commonly occur in children and young adults. IMTs display a wide morphologic spectrum, ranging from a predominantly inflammatory lesion with a paucity of spindle cells and prominent chronic inflammation and/or hyalinized stroma, to a highly cellular myofibroblastic proliferation and occasionally frankly sarcomatous neoplasm, lacking a significant inflammatory or/and stromal component. Due to its variable morphologic phenotype and lack of a consistent immunoprofile, the diagnosis of IMTs in the absence of *ALK*-rearrangements, has been often a diagnosis of exclusion with a challenging differential diagnosis including at one end of the spectrum reactive/inflammatory processes, such as the fibro-inflammatory IgG4-related disease, idiopathic retroperitoneal fibrosis, and postoperative myofibroblastic proliferations, while at the other end of the spectrum, spindle cell sarcomas with myofibroblastic or fibroblastic features. In our recent series of 62 IMTs (25 presenting in children), gene rearrangements involving kinases were found in two-thirds of cases, including *ALK* fusions in 56%, *ROS1* in 10%, and one case with *RET* rearrangement.¹⁷ In that study, pediatric IMT showed a 90% rate of kinase fusion-positive results. Furthermore, most IMT with *EML4-ALK* fusions occurred in children, including 2 newborns (range 0-39 years, mean 15). Similarly, all except one case of *ROS1*-rearranged IMTs occurred in the pediatric age group, showing slender spindle cells with distinctive long cell processes arranged in loose fascicles, with mild inflammatory component and a variably fibromyxoid stroma. *ROS1* IHC can be applied as a surrogate method for *ROS1* rearrangement screening in IMT.¹⁸ Additionally a handful of cases of IMT with *ETV6-NTRK3* fusions have also been reported.^{19,20} Importantly, a subset of IMTs with *ALK* or other kinase fusions show durable response to the ALK inhibitor Crizotinib.^{21,22}

II. NEW GENETIC ADVANCES IN FIBROBLASTIC PEDIATRIC LESIONS

1. *EGFR* ITD/MUTATION IN FIBROUS HAMARTOMA OF INFANCY

Fibrous hamartoma of infancy (FHI) is a rare benign subcutaneous soft tissue tumor, first described by Enzinger as a tumor with a distinctive tripartite histological appearance.²³ FHI is more prevalent in boys in the first two years of life. The most common anatomic sites are trunk, extremities, head and neck and genital regions. Grossly, FHI is a poorly circumscribed subcutaneous fatty mass with a mean size of 3 cm. Microscopically, FHI consists of adipose tissue intermixed with long interlacing fascicles of dense fibrous tissue surrounded by fibromyxoid nodules consisting of immature spindle cells, an architecture which gives the lesion an organoid appearance (Fig. 3).^{24,25} Apart from the typical triphasic histology, up to half of the cases have hyalinized areas with dense collagen and long slit-like pseudoangiomatous spaces. By IHC, a subset of FHI shows variable expression of smooth muscle actin and CD34, consistent with the fibroblastic nature of the tumor. FHI are benign, with a small subset showing local recurrence, likely due to incomplete primary excision, in particular of large poorly circumscribed tumors.

The genetic hallmark of FHI, recently coined by NGS and targeted Sanger sequencing, represents a somatic *EGFR* exon 20 insertion/duplication (Fig. 3), likely resulting in oncogenic EGFR activation.²⁶ Notably, immunohistochemistry applied in that study revealed a moderate degree of EGFR protein expression, particularly in the primitive cell component. A similar genotype was also reported in FHI with variant histology, including tumors with a predominant pseudoangiomatous morphology, which can mimic giant cell fibroblastoma.²⁷ No *EGFR* mutations were noted in other fibrolipomatous control cases tested.

2. *FNI-EGF* FUSION IN CALCIFYING APONEUROTIC FIBROMA

Calcifying aponeurotic fibroma (CAF) is a benign fibroblastic tumor with a predilection for the soft tissues of the hands and feet, with a peak incidence in the first decade of life.^{28,29} Boys are afflicted more often than girls. CAF is a solid, nodular subcutaneous tumor, usually less than 3 cm in size, often connected to aponeurosis, tendon sheath or joint capsule. Microscopically, the tumor is often well-circumscribed, non-encapsulated, although occasionally might have infiltrative margins. CAF has a biphasic morphology, composed of bland appearing fibroblasts arranged either haphazardly or in short storiform fascicles, which alternate with partly calcified fibrocartilage-like nodules. The latter areas show finely granular dystrophic calcifications surrounded by hyalinized collagen, with embedded epithelioid fibroblasts resembling chondrocytes, as well as scattered osteoclast-type giant cells (Fig. 3). CAF may recur locally, particularly in young children. Differential diagnosis includes palmar fibromatosis and soft tissue chondroma. The genetics of CAF were recently defined by the presence of a recurrent *FNI-EGF* gene fusion, resulting from a presumed *ins(2;4)(q35;q25)*.³⁰ The likely pathogenetic mechanism is an inappropriate expression of the biologically active portion of EGF, which can be detected immunohistochemically, driven by the strong *FNI* promoter activity.

3. *EWSR1-SMAD3* FUSION POSITIVE FIBROBLASTIC TUMOR WITH ERG POSITIVITY

An *EWSR1-SMAD3* fusion was recently identified as the genetic hallmark of a novel benign fibroblastic neoplasm with predilection for acral soft tissues.³¹ The index case occurred in the foot of an infant male, presenting as an ill-defined dermal and subcutaneous nodule with an infiltrative border. The tumor was composed of intersecting cellular fascicles of uniform plump fibroblastic-type spindle cells with bland fusiform nuclei (Fig. 3). Two additional cases presenting superficially in the foot of adult women harbored identical gene fusion and histologic appearance. However, the adult lesions shared some similarities to CAF, with a distinctive zonation pattern, with peripheral hypercellular areas transitioning to hypocellular collagenous areas, containing fine granular calcifications. Unsupervised clustering of the whole transcriptome data showed that the *EWSR1-SMAD3* fusion positive tumor clustered together with other fibroblastic tumors, including a CAF with *FNI-EGF* fusion, a lipofibromatosis lacking genetic abnormalities, and a LPF-NT with *TPR-NTRK1* fusion. Interestingly, *ERG* mRNA was strongly upregulated, finding further supported by diffuse nuclear immunoexpression of ERG protein.³¹

III. PERIVASCULAR MYOID TUMORS

The spectrum of perivascular myoid neoplasms includes myofibroma, myopericytoma, angioleiomyoma, and glomus tumor.³² It is well recognized that these tumors may have overlapping or hybrid histologic features. Their clinical presentation varies with age at diagnosis, with myopericytoma, angioleiomyoma, and glomus tumor rarely occurring in children, whereas myofibromas may present at any age. Myofibromas are either solitary or multicentric (a.k.a. myofibromatosis). Solitary (infantile) myofibroma displays a biphasic histomorphology, consisting of fascicles with myofibroblastic cells, alternating with areas of immature spindle cells associated with branching hemangiopericytoma-like vessels. Sites of predilection are the head and neck region, including tongue and mandible, and upper trunk. Bone and internal organs are often involved in myofibromatosis, in addition to soft tissue locations. Immunohistochemically, the immature spindle cells in myofibromas may express CD34, whereas myofibroblastic cells typically are actin-positive. Myofibromas with myoid or myopericytic differentiation may focally express desmin and caldesmon. The molecular genetics of perivascular myoid tumors have been only recently elucidated.

1. *PDGFRB* MUTATIONS IN MYOFIBROMA(TOSIS) AND MYOPERICYTOMA(TOSIS)

Recurrent activating mutations in the platelet-derived growth factor receptor beta gene (*PDGFRB*) are found in nearly all autosomal dominant myofibromatosis.³³⁻³⁵ In these families the *PDGFRB* R561C hot spot mutation is the most common variant, whereas the P660T and P560L variants are relatively rare. Subsequently, various activating *PDGFRB* mutations were detected in cases of nonfamilial myofibromatosis³⁶ and in the majority of sporadic myofibromas encountered in infants (75%) and adults (69%), with N666K mutation being detected in about half of the cases.³⁷ These activation mutations are mostly located in the juxtamembrane and kinase domains of the *PDGFRB* receptor and were shown to be sensitive to tyrosine kinase receptor inhibition in experimental models. In addition, a *NOTCH3* c.4556T>C mutation was

described in a family with myofibromatosis.³⁴ *NOTCH3* is a regulator of PDGFRB and both signaling pathways play an essential role in the proliferation and migration of subsets of pericytes and vascular smooth muscle cells during vascular development and angiogenesis. Not surprisingly, *NOTCH* rearrangements and fusions associated with *MIR143* have been implicated in more than half of glomus tumors; the incidence being much higher in malignant examples.³⁸ In addition to myofibroma(tosis), RNA sequencing also revealed *PDGFRB* mutations in myopericytoma and myopericytomatosis, a condition presenting in the lower extremity of adults, characterized by diffuse dermal and/or subcutaneous involvement by myopericytomatous nodules.³⁹

2. *SRF-RELA* FUSION IN CELLULAR MYOFIBROMA/MYOPERICYTOMA

SRF rearrangements, in particular *SRF-RELA* fusions, were recently described in a group of cellular myofibroblastic tumors with histologic features reminiscent of cellular myofibroma or cellular myopericytoma.⁴⁰ Most of these tumors presented in children younger than 18 years of age. The few cases with follow-up did not indicate aggressive or metastatic behavior. By IHC, these cellular myofibroblastic tumors had a smooth muscle-like phenotype with abundant expression of smooth muscle actin, desmin, and caldesmon. Microscopically, two architectural patterns were observed, one being composed of monomorphic spindle cells arranged in intersecting fascicles, while the other consisted of rounded to ovoid cells arranged in either a syncytial growth or a nested architecture around a rich capillary network. Biphasic patterns reminiscent of myofibroma were also encountered. *SRF-RELA* fusions were not encountered in classic examples of myofibroma or myopericytoma.

IV. SPINDLE CELL/SCLEROSING RHABDOMYOSARCOMA AND OTHER NOVEL MOLECULAR GROUPS

Pediatric rhabdomyosarcomas (RMS) have been traditionally divided into embryonal and alveolar subtypes, defined by their different clinical presentation, histologic appearance, and outcome. The large majority of alveolar RMS harbor *PAX3/7-FOXO1* fusions, while embryonal RMS lack a recurrent genetic abnormality. However, in the latest WHO classification, one additional independent subgroup was recognized, the so-called ‘spindle and sclerosing RMS’, which was separated from the embryonal group based on its distinctive histologic and clinical features.³² This review focuses mainly on this latter subtype, where most recent molecular discoveries have been made.

Spindle cell RMS was originally described as a rare and prognostically favorable variant of embryonal RMS in the pediatric age group, mainly presenting in young males as paratesticular tumors or in the head and neck region.⁴¹ A subsequent series of spindle cell RMS in adults suggested a more aggressive clinical course compared to pediatric cases.⁴² Histologically, spindle cell RMS is composed of monomorphic undifferentiated spindle cells arranged in long

herringbone fascicles intermixed with variable amount of collagen. A subset of these lesions shows abundant hyaline collagen deposition with a deceptively bland spindle cell appearance, being designated as sclerosing RMS. Of note both spindle and sclerosing subtypes typically lack differentiating rhabdomyoblasts or significant nuclear pleomorphism (Fig. 4). Instead, solid areas of primitive round cells, reminiscent of a solid alveolar RMS, can be noted, particularly in the setting of sclerosing subtype.⁴³ In this regard, a recent re-review of the alveolar RMS patients enrolled in COG studies from 1999-2005 found that 30% of the tumors were reclassified as either embryonal or sclerosing RMS,⁴⁴ the main pitfall being the presence of dense or sclerosing patterns. By IHC, spindle and sclerosing RMS shows reactivity for desmin and MyoD1, as well as multifocal expression of myogenin. The genetic abnormalities of spindle cell/sclerosing RMS were recently characterized, which are distinct from embryonal RMS and vary based on the age at presentation and anatomic location. Despite their overlapping morphologic features, at least three genomic groups have been defined in the pediatric age group, including: an infantile subset of spindle cell RMS harboring *VGLL2*-related gene fusions, a *MYOD1*-mutant subset commonly associated with sclerosing morphology, and a subset lacking recurrent genetic abnormalities.

1. FUSION-POSITIVE CONGENITAL OR INFANTILE SPINDLE CELL RMS.

These infantile tumors harbor recurrent gene fusions, involving critical transcriptional activators of muscle-specific genes, such as *VGLL2*, *TEAD1* and *SRF*.^{43,45} The most common variant was *VGLL2-CITED2* (Fig 4), followed by *VGLL2-NCOA2*, with rare cases displaying *TEAD1-NCOA2* and *SRF-NCOA2*. These patients present at birth or within one year of age, with predilection for trunk, and follow a favorable clinical outcome, lacking metastatic potential. The clinical behavior of fusion-positive infantile spindle cell RMS group resembles that of *ETV6-NTRK3*-positive infantile fibrosarcoma; these findings militate against their classification as a ‘high grade neoplasm’. A recent study however, suggested that *VGLL2*-fusion positive tumors might represent a biologically and pathologically more heterogeneous group of tumors than initially described.⁴⁶ Based on 7 such cases, 6 positive for *VGLL2-NCOA2* and one for *VGLL2-CITED2*, the authors describe 2 histologic patterns, including a deceptively bland ‘fibrous’ subtype and a ‘dense’ cellular subtype. Despite their positivity for myogenin and expression of muscle-related genes transcriptionally, these tumors did not cluster genomically with other RMS samples.

2. *MYOD1*-MUTANT SPINDLE CELL/SCLEROSING RMS.

The presence of transactivating *MYOD1* mutations have been described in both spindle cell and sclerosing RMS, providing a strong molecular basis in support for their classification as a single pathologic entity.⁴⁷ At the molecular level, most tumors harbor a *MYOD1* homozygous mutation in exon 1 (p.L122R) (Fig. 4), while a smaller subset shows a heterozygous genotype. *MYOD1* regulates muscle cell differentiation by inducing cell cycle arrest, a prerequisite for myogenic initiation. Mutation of Leu 122 to Arg in *MYOD1* has been shown to confer reduced transcriptional activation at *MYOD1* sites, together with enhanced binding to *MYC* sites.⁴⁸ RMS

patients with *MYOD1* mutations, with or without accompanying *PIK3CA* mutations, follow a highly aggressive course with high mortality despite multimodality therapy.^{47,49,50} In a recent study, *MYOD1* mutations were the most common genetic abnormality in pediatric spindle cell/sclerosing RMS, occurring in 64% of children beyond one year of age, and suggesting that it can be used as a molecular biomarker to stratify these high risk patients.⁴³ The head and neck location represents the most common site, occurring in one-third of patients, followed by extremities and trunk.

3. 'WILD-TYPE' SPINDLE CELL/SCLEROSING RMS

This group encompasses the 'genetically negative' group, lacking gene fusions or *MYOD1* mutations, often presenting intra-abdominally or para-testicular. This molecular-negative group is typically associated with a favorable clinical course and most likely represents a spindle cell variant of embryonal RMS.⁵¹

4. NOVEL EPITHELIOID RMS VARIANT DEFINED BY *EWSR1/FUS-TFCP2* FUSIONS.

The presence of *EWSR1/FUS-TFCP2* fusions has been recently reported in 3 tumors from young adult females occurring with predilection at skeletal sites (pelvis, chest wall, sphenoid bone).⁴⁶ The tumors were composed of a predominant epithelioid sheet-like growth, although focal areas of fascicular spindled cells were also observed. Overall histology including a high mitotic activity was in keeping with a high grade malignancy. Their immunoprofile showed positivity for desmin, MYOD1, myogenin and ALK. The patients followed a highly aggressive course, succumbing to disease in less than 6 months. These findings were further confirmed by a recent case report of a 72-year-old male with a destructive lesion of the mandible, which harbored a similar *FUS-TFCP2* fusion.⁵² However, the morphologic findings in this report appear to be distinct, the authors describing it as an unusual molecular variant of spindle cell RMS of bone, rather than having an epithelioid phenotype. Further studies are needed to establish if this new molecular signature defines a novel RMS pathologic entity or simply a genetic variant of spindle cell/sclerosing RMS with predilection for skeletal sites.

V. UNDIFFERENTIATED TUMORS WITH *BCOR* ONCOGENIC ACTIVATION – 'BCOR FAMILY OF TUMORS'

Undifferentiated sarcomas characterized by *BCOR* genetic alterations, spanning both gene fusions and internal tandem duplications (ITD), share similarities at the morphologic and immunohistochemical levels, despite the wide spectrum of clinical presentations and pathologic entities involved. *BCOR* (BCL6 interacting corepressor) is a transcriptional corepressor involved in gene suppression by either interacting with BCL6 or binding to PCGF1 (polycomb-group RING finger homologue 1) as part of a variant Polycomb Repressive Complex 1 (PRC1.1), associated with chromatin remodeling and histone modification.⁵³ *BCOR* genetic abnormalities implicated in sarcomagenesis consistently involve the last exon of *BCOR*, which encodes the

PUFD domain, responsible of anchoring the C-terminal BCOR to the PCGF1 unit of the PRC1.1 complex.⁵⁴ Thus formations of gene fusions or ITD most likely alter the binding affinity between BCOR and PCGF1, affecting the subsequent recruitment of KDM2B and downstream gene regulation.⁵⁵ Regardless of the specific gene alterations, BCOR family of tumors show significant upregulation of *BCOR* and *SATB2* mRNA, resulting into protein overexpression and immunopositivity for BCOR and SATB2, which can be used as reliable diagnostic markers.⁵⁶

1. INFANTILE UNDIFFERENTIATED SOFT TISSUE TUMORS WITH *BCOR*-INTERNAL TANDEM DUPLICATIONS

In a recent study about half of infantile soft tissue undifferentiated round and spindle cell sarcomas harbored recurrent *BCOR* exon 15 ITD, with rare cases showing instead *YWHAE-NUTM2B* fusions.⁵⁷ The cohort included 7 boys and 4 girls, ranging in age from 8 days to 11 months, involving the soft tissues of the trunk, retroperitoneum/pelvis and head and neck. Histologically, the tumors were composed of primitive round to ovoid cells arranged in vague nests and compartments delineated by a delicate arborizing vascular network or cellular fibrous septa (Fig. 5). A subset of tumors displayed extensive myxoid and cystic changes, highly reminiscent of the previously described entity ‘primitive myxoid mesenchymal tumor of infancy (PMMTI)’.⁵⁸ Indeed further molecular analysis of cases previously designated as PMMTI showed similar *BCOR* ITD abnormalities, in keeping with a morphologic spectrum. Moreover, as *BCOR* ITD alterations were described recently as the leading pathogenetic mechanism of clear cell sarcoma of kidney (CCSK),⁵⁹ a comparative histologic and genomic analysis of the 2 tumor types showed significant overlap with the infantile soft tissue tumors harboring a similar genotype.⁵⁷ These findings suggest that *BCOR*-ITD defines a novel pediatric undifferentiated sarcoma, which encompasses tumors previously designated as unclassified infantile primitive sarcomas, PMMTI and CCSK (Fig. 5). This unified disease entity shows a homogenous immunoprofile, with strong and diffuse positivity for BCOR and SATB2,^{57,60} as a result of oncogenic *BCOR* gene upregulation. Further studies are needed to better define their clinical behavior and treatment; particularly if these tumors should be managed with undifferentiated sarcoma or CCSK type therapeutic strategies.

2. *BCOR-CCNB3* FUSION POSITIVE PRIMITIVE SARCOMAS

BCOR-CCNB3 fusion positive sarcoma was first described by Pierron et al.⁶¹ as a small blue round cell malignancy with overlapping demographics and morphology with Ewing sarcoma, but showing distinct copy number abnormalities. The genetic hallmark consists of a paracentric inversion on the short arm of chromosome X, resulting in the fusion of 2 nearby genes, *BCOR* and *CCNB3* (10 Mb apart), leading to *CCNB3* overexpression. The initial report suggested a strong predilection for male children and skeletal anatomic sites, while two subsequent smaller studies showed a more even distribution between bone and soft tissue locations.^{62,63} In a recent comprehensive analysis of 36 cases, the clinicopathologic features were further characterized, showing a median age at diagnosis of 15, a striking male predominance (M:F=31:5), and a slight

predilection for bone anatomic sites.⁶⁴ Kidney was the only visceral location, with one of these 2 cases presenting in a 12 year-old male with a markedly cystic lesion.⁶⁵ Histologically, the tumors displayed a primitive and monomorphic cytology, composed of variable proportions of round to spindle cells, arranged in solid sheets or short fascicles, in a sparse myxoid or collagenous stroma. In fact, tumors showed significant morphologic overlap with other primitive tumors with *BCOR* genetic abnormalities. Immunohistochemically, tumors showed strong and diffuse expression for BCOR and SATB2 (Fig 5).⁶⁴ Follow-up information revealed a similar outcome to Ewing sarcoma, with a 5-year overall survival of 72%, but significantly more favorable than *CIC-DUX4* sarcomas. In keeping with the distinct clinical behavior, the BCOR family of tumors (including BCOR-CCNB3, BCOR-ITD) had similar expression signature clustering together by unsupervised clustering of RNA sequencing, separate from other primitive sarcomas, such as Ewing, *CIC-DUX4* and synovial sarcomas.⁶⁴

3. RELATED TUMORS WITH BCOR UPREGULATION

Rare cases with alternative *BCOR* fusions have also been reported, including *BCOR-MAML3* and *ZC3H7B-BCOR*, which result from more conventional inter-chromosomal translocations.⁶⁶ Tumors with these fusions were shown to share significant morphologic and immunohistochemical features with other members of the BCOR-family tumors.^{56,64} Interestingly, about of half of synovial sarcomas also show BCOR overexpression, including rare cases with *SS18L1-SSX1* fusions.^{64,67} Moreover, in addition to the common BCOR overexpression, it was recently recognized the close histologic overlap between tumors with BCOR genetic abnormalities, in particular BCOR-CCNB3, and synovial sarcomas.^{56,64,67}

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FIGURE LEGENDS

Fig. 1. Pediatric spindle cell sarcomas resembling infantile fibrosarcoma with alternative *BRAF* and *NTRK1* gene fusions. Morphologic spectrum showing monomorphic spindle cell fascicular growth across various clinicopathologic features and gene fusions, including a retroperitoneal lesion in a 16/F showing a *SEPT7-BRAF* fusion (A, see E diagram); paraspinal mass in a 18 months old male with a *CUX1-BRAF* fusion (B); retroperitoneal tumor in a 7 week male showing a *TPM3-NTRK1* fusion (C); foot lesion in a 1 year-old male with an *EML4-NTRK3* (D); diagrammatic representation of the *SEPT7-BRAF* intra-chromosomal translocation, resulting in the fusion of *SEPT7* exon 11 to *BRAF* exon 11 (E).

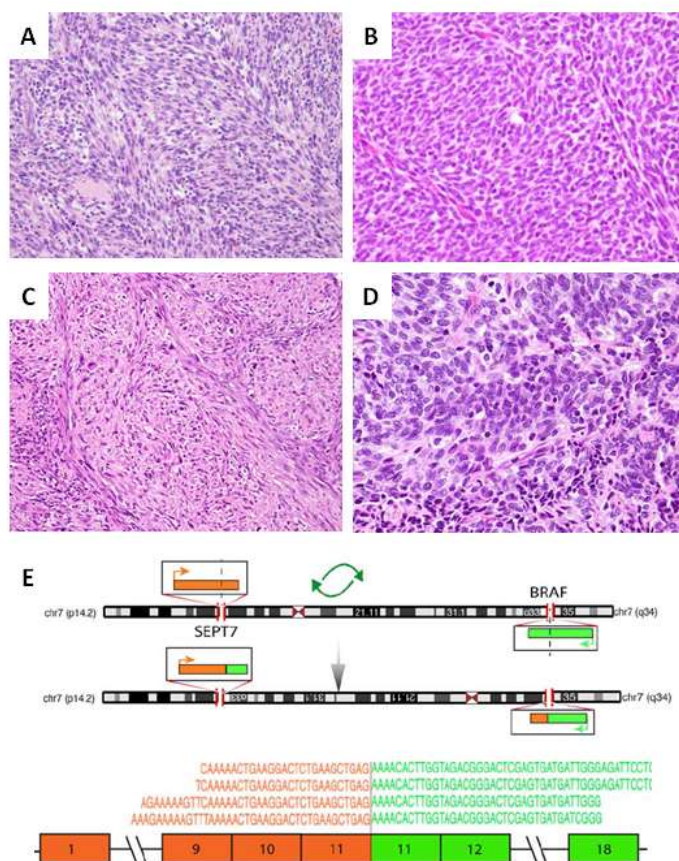
Fig. 2. Recurrent *NTRK1* fusions define lipofibromatosis-like neural tumors. Microscopic appearance of a hand soft tissue lesion in a 7 year-old male showing bland short spindle cells infiltrating within adipose tissue (A). The tumor cells were diffusely positive for NTRK1 (B). The tumor harbored a *TPR-NTRK1* fusion by RNA sequencing; diagrammatic representation showing the intra-chromosomal break and inversion of the two genes, 29 Mb apart, with opposite directions of transcription (C).

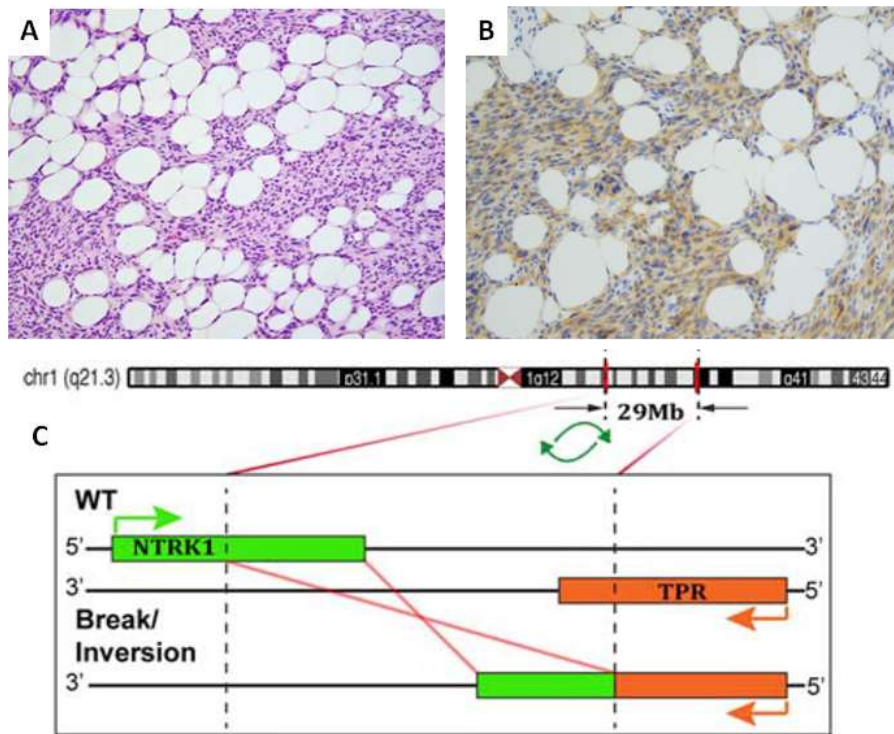
Fig. 3. Benign fibrous lesions of childhood. Microscopic appearance of a **fibrous hamartoma of infancy (FHI)** arising in the left arm in a 11 month-old male showing the typical triphasic pattern, composed of adipose tissue intermixed with long interlacing fascicles of dense fibrous tissue and fibromyxoid nodules consisting of immature spindle cells (A,B). Microscopic appearance of **calcifying aponeurotic fibroma (CAF)** arising in the hand of a 7 year-old male showing bland fibroblastic type cells embedded in a hyalinized stroma and associated with dystrophic calcifications (C). Whole transcriptome RNAsequencing of the FHI case above showing an *EGFR* internal tandem duplication (arrow, CRA unpublished results) (D), which resulted in marked upregulation of the *EGFR* mRNA in this case, compared to other pediatric fibroblastic and myofibroblastic lesions (E). CAF case from above harbored a *FNI-EGF* fusion by RNA sequencing, resulting in marked upregulation of *EGF* mRNA, compared to other soft tissue tumors (F). Infantile fibroblastic tumor displaying *EWSR1-SMAD3* (G, H), resulting in ERG overexpression (I).

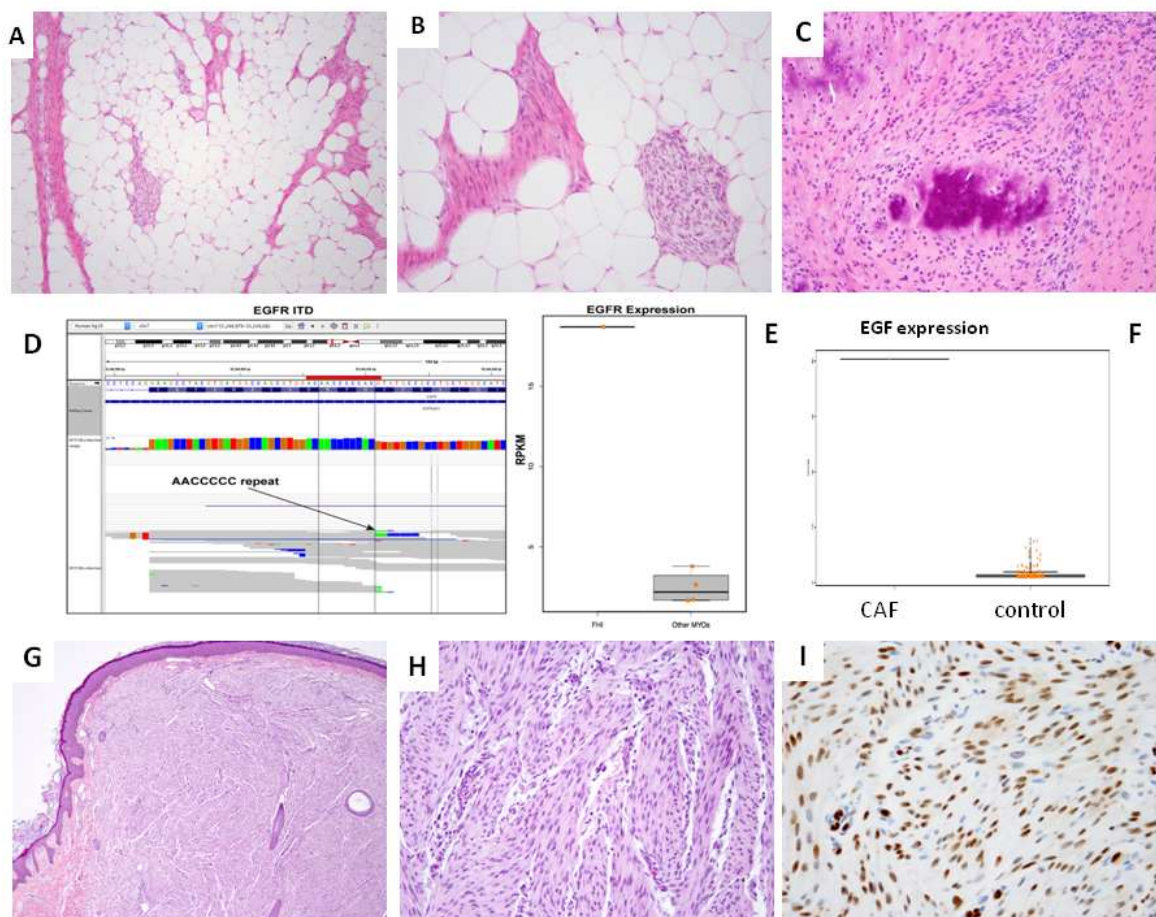
Fig. 4. Spindle cell and sclerosing RMS molecular classification. **A.** *VGLL2-CITED2* fusion positive congenital spindle cell RMS diagnosed at birth in the back of a baby-female was composed of long intersecting fascicles in a herring-bone pattern of growth; **B.** *MYOD1*-mutant spindle cell RMS arising in the head and neck of a 9 year-old male showing monomorphic spindle cells arranged in vague fascicles; **C.** Sclerosing RMS from the infratemporal fossa tumor arising in a 15 year-old female, showing monomorphic cytomorphology arranged in distinct pseudovascular spaces, separated by sclerotic stroma and showing **D.** diffuse and strong MYOD1 expression. **E.** Diagrammatic representation of a intra-chromosomal inversion in 6q22.1 resulting

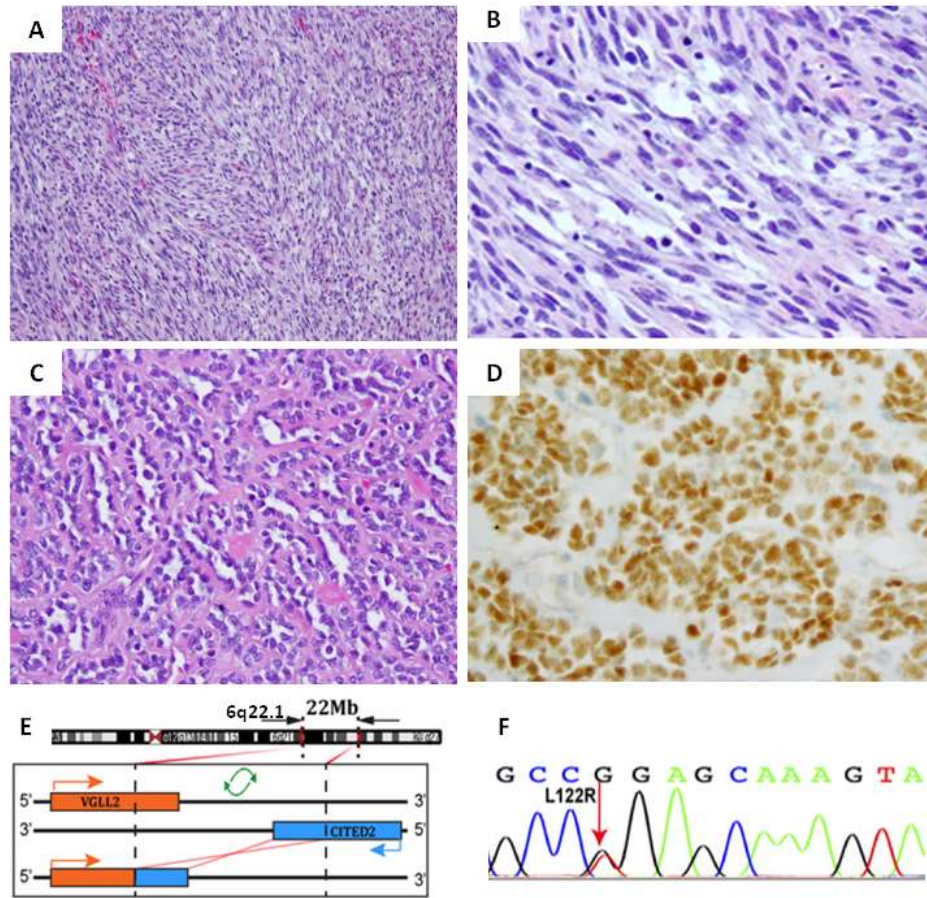
in a *VGLL2-CITED2* fusion; F. Hot spot L122R exon 1 *MYOD1* mutation; arrow indicates a heterozygous genotype.

Fig. 5. BCOR family of tumors show significant morphologic overlap including a primitive round to ovoid phenotype arranged in compartments delineated by fibrovascular septae. The members of this family include: (A) infantile soft tissue undifferentiated round cell sarcoma with *BCOR* ITD; (B) clear cell sarcoma of the kidney with *BCOR* ITD; round cell sarcomas with either *BCOR-MAML3* (C) or *BCOR-CCNB3* (D) fusions. The tumors also have in common strong and diffuse reactivity for BCOR (E) and SATB2 (F), regardless of the genetic abnormality. (E,F, *BCOR*-ITD positive retroperitoneal tumor in a 6 month-old male).









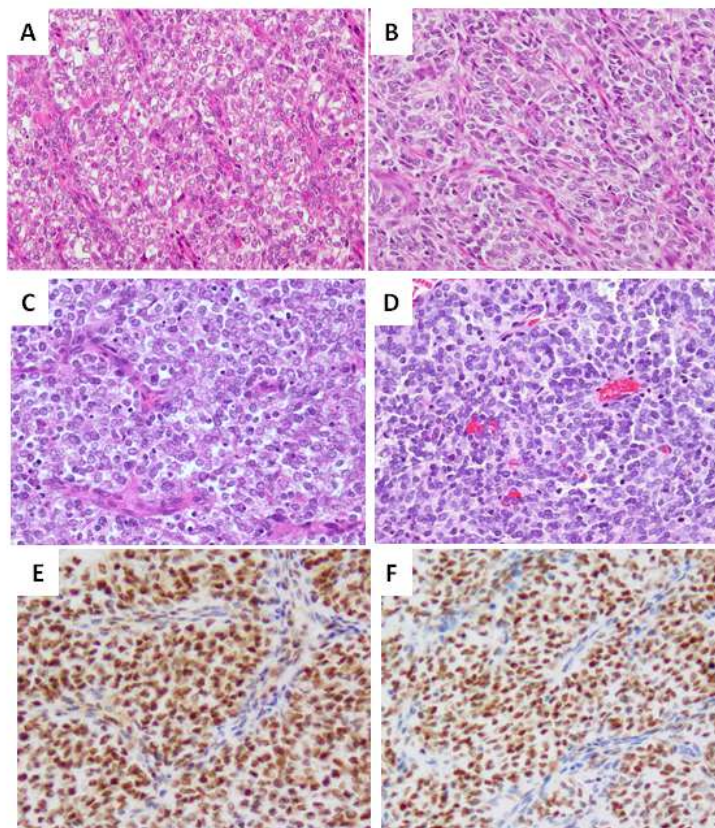


Table 1. Recently described fusion genes in pediatric sarcomas resembling infantile fibrosarcoma

Gene abnormality/ fusion	Tumor Site	Age	Sex	Reference #
<i>EML4-NTRK3</i>	foot	21 mo	M	#6
<i>EML4- NTRK3</i>	retroperitoneum	7 mo	F	#5
<i>EML4- NTRK3</i>	Lung metastasis	1 yr	F	#5
<i>EML4- NTRK3</i>	NA	24 days	F	#8
<i>SEPT7-BRAF</i>	Retroperitoneum	16 yrs	F	#6
<i>CUX1-BRAF</i>	thoracic spine	18 mo	M	#6
<i>BRAF</i> rearranged	Pelvis	3 yrs	F	#6
<i>BRAF</i> rearranged	Pelvis	6 mo	F	#6
<i>BRAF</i> rearranged	Thigh	2 days	M	#6
<i>BRAF</i> intragenic deletion	NA	79 days	M	#8
<i>BRAF</i> intragenic deletion co-existing	NA	107 days	F	#8

<i>ETV6-NTRK3</i>				
<i>BRAF</i> intragenic deletion co-existing <i>ETV6-NTRK3</i>	NA	NA	NA	#8
<i>TPM3-NTRK1</i>	Retroperitoneum	7 weeks	M	#6
<i>LMNA-NTRK1</i>	Soft tissue, NOS	1 yr	M	#7
<i>SQSTM1-NTRK1</i>	Soft tissue, NOS	0 yr	F	#7
<i>TFG-MET</i>	Pelvic mass	4 mo	F	#9

Mo, months; yrs, years; M, male; F, female, NA, not available, NOS, not otherwise specified